

REVIEW

MINERALS IN THE HUMAN BODY

## Mineral precipitation and dissolution in the kidney

MICHAEL G. HILL<sup>1,\*</sup>, ERICH KÖNIGSBERGER<sup>1</sup>, AND PETER M. MAY<sup>1</sup>

<sup>1</sup>Chemical and Metallurgical Engineering and Chemistry, Murdoch University, 6150 Murdoch, Western Australia, Australia

ABSTRACT

The formation of kidney stones is a significant human health problem. Calcium minerals are involved in a majority of these stones. Despite much research, the processes involved in stone formation remain poorly understood and, hence, reliable procedures for preventing their formation have yet to be developed. However, recent advances point to some key steps in mineral formation and transformation involving calcium phosphates, which can help to illuminate these issues. A computer model has been developed to express the current status of literature data succinctly and to illustrate that computer modeling is a powerful tool for calculating mineral solubilities and for providing insight into the processes involved. Determining the nature of the initial solid phase of calcium phosphate formed is evidently important.

**Keywords:** Kidney disease, urolithiasis, apatite, brushite, whewellite, weddellite, review article

### INTRODUCTION

Urolithiasis denotes the pathological crystallization of minerals that are deposited in the form of calculi or “stones” in the urinary tract, especially in the kidney. In contrast to the biologically controlled formation of bone and teeth, urolithiasis is a spontaneous process resembling the formation of minerals in low-temperature, aqueous geochemical environments. This review explores the thermodynamic and kinetic aspects of mineral-urine interactions, together with pathological preconditions of urolithiasis. Various calcium phosphate minerals are crucially involved in kidney stone pathology, but many chemical and mineralogical issues relating to them remain unclear. We summarize what is currently known and identify the most important areas for future work. Progress is unlikely unless current understanding can be made more quantitative.

Kidney stone formation is a worldwide problem (Linder and Little 1986; Grases et al. 1999; Moe 2006) and is very painful (Grases et al. 1998; Thomas and Hall 2005). There is a high economic cost associated with the condition as a result of hospitalization and days taken off work (Linder and Little 1986; Grases et al. 1998, 1999; Parks and Coe 1996). Although surgical treatments have improved, there is a high, and increasing, incidence of the pathology (Romero et al. 2010; Tiselius 2011b). Despite much research, the underlying causes are still not well understood; prevention has therefore proved difficult (Söhnle and Grases 1995; Grases et al. 1998; Grases and Costa-Bauza 2006; Evan et al. 2015; Tiselius 2015).

Some risk factors, however, are well known. Incidence is age and gender dependent, being twice as common in males than in females (Hesse et al. 1986; Moe 2006; Hughes 2007; Romero et al. 2010; Tiselius 2011b), with a peak age of presentation at

20 to 50 yr (Robertson et al. 1981; Hesse et al. 1986; Hughes 2007). Dietary factors are significant, especially increasing risk are diets high in animal protein (Abdel-Halim 2005; Tiselius 2011b) and fat (Tiselius 2011b). Insufficient fluid intake, resulting in a more concentrated urine significantly exacerbates the problem (Tiselius 2011b). Obesity is another well known risk factor (Abdel-Halim 2005; Hughes 2007; Romero et al. 2010; Tiselius 2011b; Rendina et al. 2013). The environment also has an effect: risk is increased for those living in hot climates and in periods of hotter weather (Soucie et al. 1994; Moe 2006; Hughes 2007; Romero et al. 2010). Genetic influences are known to be important and differences have been noted in rates of urolithiasis between different racial groups. Incidence and prevalence is highest in Caucasians, decreasing in Hispanics and Asians and lowest in Africans (Soucie et al. 1994; Hughes 2007; Romero et al. 2010; Moran 2014). In fact, kidney stones are very rare in most of Sub-Saharan Africa (Kumar and Muchmore 1990; Rodgers 2006).

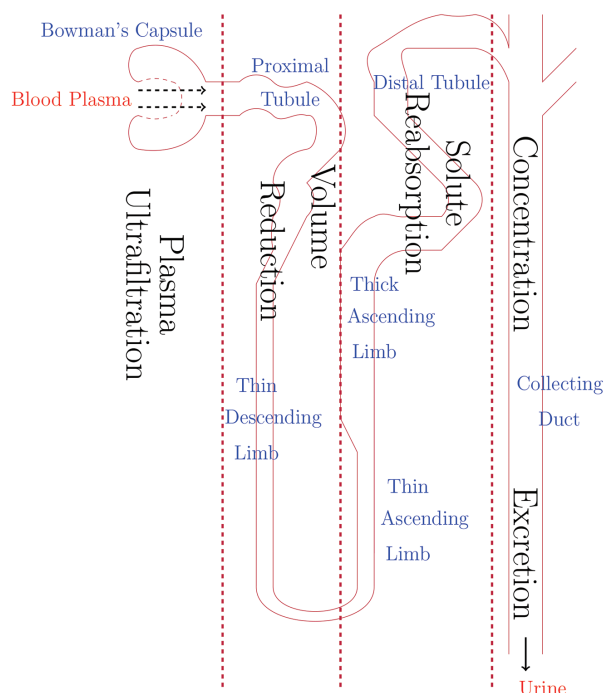
### PHYSIOLOGY

The kidneys perform the vital function of removing unwanted substances from the blood. To understand kidney stone formation it is necessary to consider first the processes of filtration and reabsorption. The balance between these two plays a key role in the potential nucleation of stone forming minerals. Filtration starts with an unselective separation, where the fluid that is blood plasma passes through an ultrafiltration membrane into the tubules of the kidney. This is then followed by (1) a selective reabsorption process, in which metabolically useful substances are returned from the filtrate back into the blood, and (2) secretion, in which unwanted substances are transferred into the fluid in the tubule, and thus ultimately become excreted in the urine.

The basic functional unit of the kidney is called a nephron. A nephron is a tube, through which flows the fluid being processed by the kidney. Each nephron consists of several sections for add-

\* E-mail: m.hill@murdoch.edu.au

Special collection papers can be found online at <http://www.minsocam.org/MSA/AmMin/special-collections.html>.



**FIGURE 1.** Schematic depicting the nephron structure and function. (Color online.)

ing or removing substances from the fluid in the tube to perform the overall extraction of urine from the blood. The most important sections for present purposes are the Bowman's capsule (BC), the proximal tubule (PT), the loop of Henle (LH), the distal tubule (DT), the collecting tubule (CT), and the collecting duct (CD). The loop of Henle is made up of a thin descending limb, a thin ascending limb and a thick ascending limb, as shown in Figure 1. Nephrons vary in length. The "long" ones have a longer loop of Henle and there will be some differences between the composition of the fluid in long and short nephrons. The output from several collecting ducts flows through the duct of Bellini, which is located in a papilla. The papillae protrude into a calyx (CX), which is a space where urine collects before exiting the kidney via the ureter (Bell et al. 1968; Guyton and Hall 2000; Kerr 1999; Atherton 2006b).

Although most of the filtrate entering the Bowman's capsule is reabsorbed, the reabsorption occurs unevenly along the length of the nephron (Atherton 2006a). Some segments reabsorb more water than solutes, and reabsorption of the solutes takes place to varying extents in different sections (Guyton and Hall 2000). This results in marked changes in solution composition and concentration as the fluid flows along the nephron (Asplin et al. 1996). As a result of the depletion of water, the solutes become more concentrated and in certain cases can become increasingly supersaturated with respect to various minerals.

The final result of the process is a solution containing all the substances to be excreted emerging at the urine-forming end of the kidney tubules.

Both the composition and daily volume of urine are very variable, both inter-individual and intra-individual (Saude et al. 2007). The pH of urine also varies from around 4.8 to 7.2

(Kok 1997). Concentrations depend on daily urine volume that can vary significantly. Figures for typical daily urine volume range from 0.99 to 2.3 L (Diem and Lentner 1970; Taylor and Curhan 2007; Eisner et al. 2010; Taylor et al. 2010). The values in Table 1 have been calculated by dividing the average value in mmol per 24 h by the volume to obtain concentration values for normal subjects.

## MINERALOGY

Minerals often occur naturally within biological structures. Multicellular entities are frequently made up of soft tissue supported by hard structures. In the case of vertebrates, these hard structures are normally composed of minerals, and biological mechanisms are generally required to construct and maintain these structures. Pathological calcifications, as in the formation of kidney stones and calculi formed in other parts of the body such as the gall bladder, pancreas, and salivary glands, may or may not involve active biological processes.

The minerals of particular relevance to this review are apatite, brushite, octacalcium phosphate, whewellite, and weddellite. Apatite comprises a group of minerals with the general formula  $\text{Ca}_5(\text{PO}_4)_3(\text{F}, \text{Cl}, \text{OH})$  (Tiselius 2011b). The minerals hydroxyapatite (hereafter abbreviated as HAP) and fluoroapatite are found ubiquitously in the body as part of the building blocks of bones and teeth (Söhnle and Grases 2011). As a result of the need to form these structures, blood plasma, and many other biofluids, are supersaturated with hydroxyapatite (Taunton et al. 2010; Söhnle and Grases 2011; Holt et al. 2014). Calcium compounds predominate in the majority of kidney stones; 85% of all kidney stones contain calcium salts. Most (about 80%) have calcium oxalate as the major component (Grases et al. 1999; Tiselius 2011b). Other stones formed are typically either calcium phosphate or mixed calcium oxalate/calcium phosphate (Coe et al. 2011).

As well as being absorbed from food, oxalate (like uric acid) is a metabolic end product (Williams 1978; Knight et al. 2006). An important function of the kidneys is therefore to excrete oxalate from the body. Given the well known insolubility of many oxalate salts, this introduces a range of possible precipitates. The calcium oxalate compounds predominantly found in kidney stones are whewellite (calcium oxalate monohydrate), and weddellite (calcium oxalate dihydrate). Calcium oxalate has three different crystal forms—the monohydrate (COM), the dihydrate (COD), and the trihydrate (COT). The literature frequently describes the monohydrate as the most stable compound, whereas

**TABLE 1.** Substance concentrations in urine

Substance	Selected concentration (mmol/L)	Selected reference	Range (mmol/L)	References
$\text{Na}^+$	151	A	45–582	B
$\text{K}^+$	32.0	A	2–260	B
$\text{Ca}^{2+}$	2.25	A	0.5–7.5	B
$\text{Mg}^{2+}$	3.35	A	0.5–12.5	B
$\text{PO}_4^{3-}$	19.9	A	5–75	B
$\text{Cl}^-$	104.9	A	118.2–236.5	C
Oxalate	0.108	A	0.1–1	B
Sulfate	12.2	D	14.8–34.5	C
Citrate	2.0	A	0.1–7.5	B
Urea	338.3	C	206.7–469.2	C

Note: A = Rodgers et al. (2006), B = Kok (1997), C = Diem and Lentner (1970), D = Siener et al. (2004).

the trihydrate is considered to be metastable and the dihydrate unstable (Tomazic and Nancollas 1980; Grases et al. 1998; Rodgers et al. 2011). This is probably due to the fact that COD cannot be precipitated from solutions that contain only calcium and oxalate ions (Tomazic and Nancollas 1980). However, COD can be precipitated from artificial and real urine and consequently often appears in kidney stones (Werness et al. 1979; Tomazic and Nancollas 1980). The solubilities of these three hydrates follow the order  $COM < COD < COT$  (Streit et al. 1998). As a result, solutions saturated with either COD or COT are supersaturated with respect to COM. Both COT and COD transform into COM (Tomazic and Nancollas 1980).

### KIDNEY STONE FORMATION

The passage of fluid through the kidney causes significant changes in concentration and hence also ionic strength (Bell et al. 1968; Guyton and Hall 2000; Atherton 2006a). These changes, which can potentially result in supersaturation, are illustrated in Figures 2, 3, 4, and 5 (Asplin et al. 1996; Hojgaard and Tiselius 1999; Kok 1997; Rodgers et al. 2011; Tiselius et al. 2009), showing plots of published values of calcium, oxalate, phosphate, and pH in different nephron segments. Kok gives probable ranges, shown as min and max in the plots. In fact, most urine samples are always supersaturated with respect to calcium oxalate and

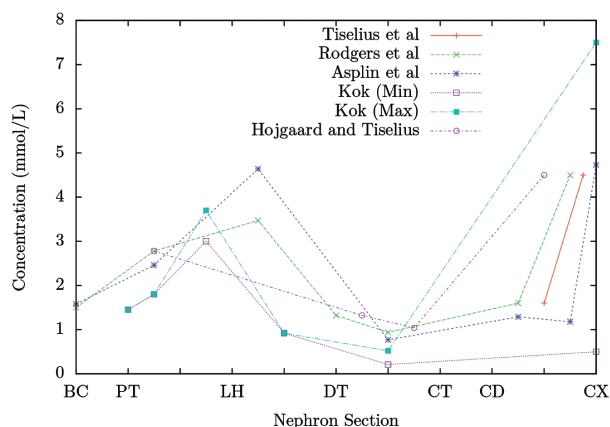


FIGURE 2. Calcium concentrations in the nephron. (Color online.)

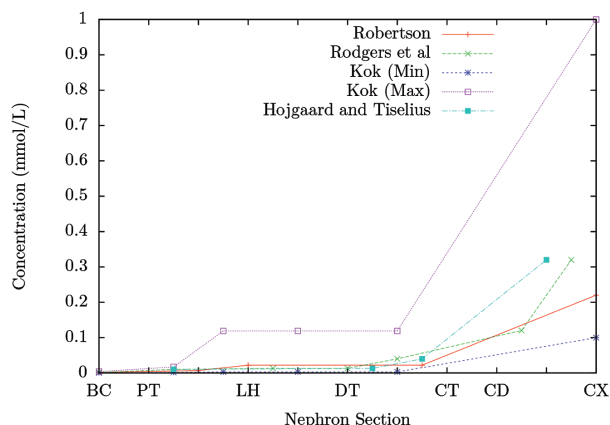


FIGURE 3. Oxalate concentrations in the nephron. (Color online.)

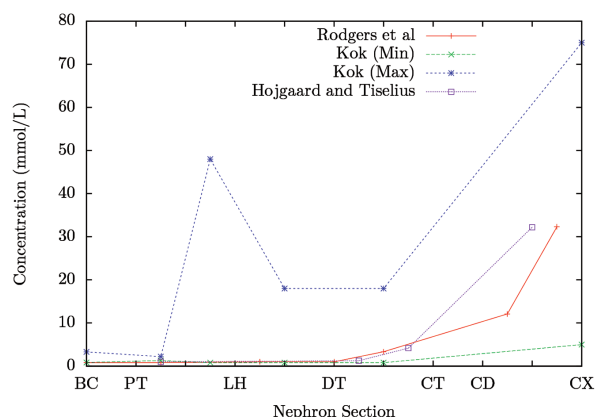


FIGURE 4. Phosphate concentrations in the nephron. (Color online.)

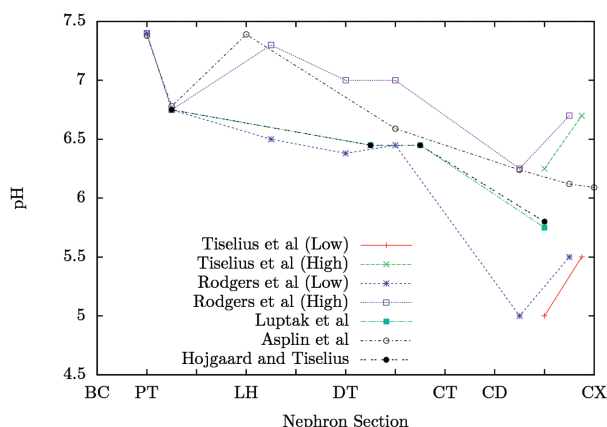


FIGURE 5. The pH variation in the nephron. (Color online.)

the calcium phosphates (Asplin et al. 1996; Grases et al. 1999).

It is known that hydroxyapatite is supersaturated throughout the length of the nephron and that there is a risk of calcium phosphate precipitation both in the ascending limb of the loop of Henle and the distal tubule (Tiselius 2011b). Calculations have shown that precipitation of hydroxyapatite can cause the other salts to become unsaturated (Rodgers et al. 2011). However, it is not known which phase of calcium phosphate is the first to precipitate (Tiselius 1997a). Our suggestion is based on Ostwald's Rule of Stages, which holds that the formation of the least stable phases precedes the thermodynamically stable phase (Söhnel and Grases 2011; Sawada 1997). This identifies the first substance to precipitate in the formation of hydroxyapatite as one of (1) amorphous calcium phosphate (ACP), having the formula  $Ca_3H_3(PO_4)_2 \cdot nH_2O$ , (2) octacalcium phosphate (OCP),  $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ , or (3) brushite (Bru),  $CaHPO_4 \cdot 2H_2O$  (Luptak et al. 1994; Asplin et al. 1996; Tiselius 1997a; Grases et al. 1997; Söhnel and Grases 2011). Knowing this initially formed phase would obviously be important in establishing how the process of kidney stone formation begins.

Urinary supersaturation with calcium oxalate monohydrate is apparently never sufficient to result in homogeneous nucleation; thus, heterogeneous nucleation must be taking place on some

nucleating substrate (Söhnel and Grases 1995; Grases et al. 2012). Hydroxyapatite, brushite, and uric acid are all likely candidates as substrates for calcium oxalate monohydrate nucleation (Robertson et al. 1981; Söhnel and Grases 1995; Tiselius 1997a; Højgaard and Tiselius 1999; Tiselius et al. 2009; Grases et al. 2012).

Most calcium oxalate stones contain a small proportion of calcium phosphate, often in the core of the stone, indicating that calcium phosphate is a common initial crystal phase (Tiselius 2011b; Højgaard and Tiselius 1999). Recent work has suggested that calcium oxalate stone formation is based on calcium phosphate precipitation higher up in the nephron, which highlights the importance of understanding the particular mechanism involved (Tiselius 2011a, 2015; Coe et al. 2011). High levels of supersaturation of calcium phosphate and higher pH can be found in the ascending limb of the loop of Henle and the distal tubule, especially in the long nephrons, which may in particular result in the precipitation of calcium phosphate (Tiselius 2011a; Rodgers et al. 2011). Precipitated calcium phosphate may then either continue to move along in the nephron tubule, or be internalized by the nephron cells, in what appears to be a defense mechanism, hence building up solid in the interstitial tissue (Tiselius 2011a). This precipitated calcium phosphate in the interstitial tissue acts as a precursor of “Randall’s Plaque” (Tiselius 2011a), which is a result of tissue damage that is most likely associated with oxidative stress (Khan and Canales 2015; Grases et al. 2015, 2016). Following loss of the normal urothelial covering of the renal papilla, the calcification of the interstitial tissue at the end of the nephron becomes exposed to urine, resulting in the formation of Randall’s Plaque (Evan 2010). There is thus strong evidence linking the presence of Randall’s Plaque to the formation of attached calcium oxalate papillary kidney stones (Coe et al. 2011; Evan et al. 2015) since almost all calcium oxalate stones show some signs of attachment (Coe et al. 2011). In most cases the point of attachment is the papilla where the protective glycosaminoglycan layer becomes damaged or defective (Söhnel and Grases 1995). These glycosaminoglycan layers have strong anti-adherent properties (Coe et al. 2011) so most calcium oxalate stones seem to be formed on Randall’s Plaque instead. Indeed, the conditions required for the formation of the most common type of stone are the presence of Randall’s Plaque and damage to the protective glycosaminoglycan layer (Tiselius 2011b, 2011a).

When calcium phosphate crystals are transported further along in the nephron tubule, the influence of pH change becomes important. If the pH is sufficiently low in the collecting duct, the calcium phosphate that has remained within the nephron tubule dissolves and brings about sufficiently high levels of calcium and oxalate concentration for crystal nucleation to occur (Kok 1997; Højgaard and Tiselius 1999). In the case where all of the calcium phosphate crystals dissolve, the resultant stone will be pure calcium oxalate, but a mixed stone results where some of the calcium phosphate remains undissolved. Whether, and how, the initial calcium phosphate precipitation can be counteracted is not yet known but has become an active focus of research (Tiselius 2011b).

Besides the Randall’s Plaque mechanism, there are two hypotheses to explain the formation of the initial entity that may lead to the formation of a kidney stone (Kok and Khan 1994). In one model, the stone starts to grow as a free particle within the

fluid in the kidney, and in the other the particle is attached from the outset to the wall of a duct within the kidney. Finlayson and Reid (1978) developed a quantitative model to describe fluid flow through the kidney and concluded that it was not possible for a kidney stone to form from a free particle. Kok and Khan (1994) examined the issue by updating the Finlayson and Reid model with more accurate data on nephron dimensions, differences between long and short nephrons, taking into account varying levels of oxalate concentration and considering the effect of crystal agglomeration, which had been left out of the original model. This study concluded that it could be possible for a particle to grow large enough to become trapped within the transit time of fluid through the nephron provided crystal size is increased by agglomeration. Robertson (2004) further enhanced the model of Kok and Khan by including the effects of drag on fluid and particles traveling close to the wall and gravity acting on particles in upward draining nephrons. The results in this case indicated that even without agglomeration the particle may still become large enough to become trapped within the lumen before reaching the end of the nephron. In the alternative “fixed particle model,” crystals become attached, usually due to renal cell injury, at the opening of the duct of Bellini, where they may subsequently grow into the so-called “Randall’s Plugs” that obstruct the lumen of the nephron and result in stones often projecting into a minor calyx (Evan 2010). The formation of Randall’s Plugs generally requires abnormally high supersaturation with respect to HAP and COM (Khan and Canales 2015).

People who suffer from calcium phosphate stones have been found to have decreased calcium reabsorption, as well as decreased  $\text{HCO}_3^-$  reabsorption in the thick ascending limb of the loop of Henle resulting in a higher pH in the distal parts of the nephron (Coe et al. 2011). As calcium phosphate precipitates only at high pH, this leads to calcium phosphate crystals being preferentially formed in the collecting ducts. It has been shown that these stones can be almost completely made up of calcium phosphate (Tiselius 2011b).

Those who suffer from calcium oxalate stones have been found to have decreased calcium reabsorption in the proximal tubule of the nephron (Coe et al. 2011). This results in high calcium concentrations within the loop of Henle and hence increased entry of calcium into the medullary interstitium and likelihood of calcium crystal nucleation in the thin limb basement membranes. The formation of Randall’s Plaque is thus accelerated.

### THE ISSUE OF SUPERSATURATION

Supersaturation with respect to the stone constituents is a requirement for stone formation (Robertson and Nordin 1976; Finlayson 1978; Grases et al. 1999; Tiselius 2011a). The composition of stones formed have been found to correspond to the supersaturation levels in the urine of the patient (Parks et al. 1997). Thus, knowledge of the state of saturation of various minerals in the ultrafiltrate as it passes through the nephron is evidently essential for an understanding of the genesis of kidney stones.

Urine is always supersaturated with respect to calcium oxalate (Robertson and Nordin 1976; Luptak et al. 1994). In the case of the calcium phosphates urine supersaturation is not as frequent (Robertson and Nordin 1976) and depends on higher pH levels (Tiselius 2011b).



Generally speaking, several carbonates (particularly calcium carbonates) in biofluids appear to be supersaturated *in vivo*. However, these calcium carbonates have not been found in kidney stones, even though they are known to form sometimes in other organs—for example, they can occur in pancreatic, salivary, and gall bladder stones, where vaterite, the least stable of these minerals has been found (Königsberger and Königsberger 2006). One possible explanation for this difference is the more acidic pH of urine but a complete understanding of these observations awaits elucidation.

The calcium oxalate hydrates are sparingly soluble substances (Königsberger and Königsberger 2006). The results of experiments to determine the solubility of sparingly soluble salts can be influenced by numerous factors including the techniques used to approach equilibrium between solid and solution and physical characteristics of the sample, affecting particle size for example (Gamsjäger and Königsberger 2003). Accurate measurement of the solubility of these salts is therefore difficult: published values of their solubilities are accordingly rather variable (Hodgkinson 1980; Königsberger and Tran-Ho 1997, Hummel et al. 2005).

The solubility products of sparingly soluble electrolytes are frequently measured as conditional solubility constants, or *concentration products* ( $K_{sp}$ ), at constant ionic strength  $I$  (Gamsjäger and Königsberger 2003). These values are functions of  $I$  and show specific ion effects at higher  $I$  (Fig. 6). In a recent review (Hummel et al. 2005), solubility products for calcium oxalates have been critically evaluated and extrapolated to  $I = 0$  (infinite dilution) using the SIT approach for the calculation of activity coefficients (see section below). In addition to the increase of CaOx solubility products with ionic strength as an effect of changing activity coefficients, Figure 6 compares selected experimental data for NaCl and KCl background electrolytes with recent critical evaluations (Hummel et al. 2005). A list of some values of solubility products at  $I = 0$  ( $K_{sp}^0$ ) published for the calcium oxalate hydrates is also shown in Table 2.

The nature of urine increases the achievable supersaturation of the salts of interest well above the measured experimental value for solubility of sparingly soluble salts. Many ions present in urine, such as  $Mg^{2+}$ , citrate, and  $HPO_4^{2-}$ , increase the solubility of CaOx by forming complexes with either the  $Ca^{2+}$  or the

**TABLE 2.** Published values of CaOx solubility products at 37 °C

Hydrate	$-\log K_{sp}^0$	Reference
COM	8.65	Finlayson et al. (1990)
COM	8.55	Daniele et al. (1985)
COM	8.65	Streit et al. (1998)
COM	8.65	Hodgkinson (1980)
COD	8.30	Finlayson et al. (1990)
COD	8.17	Streit et al. (1998)
COT	8.09	Finlayson et al. (1990)
COT	8.02	Streit et al. (1998)

$C_2O_4^{2-}$  ions (Hodgkinson 1980; Gutzow et al. 1993; Streit et al. 1998). Such complex formation generally results in an additional increase in solubility.

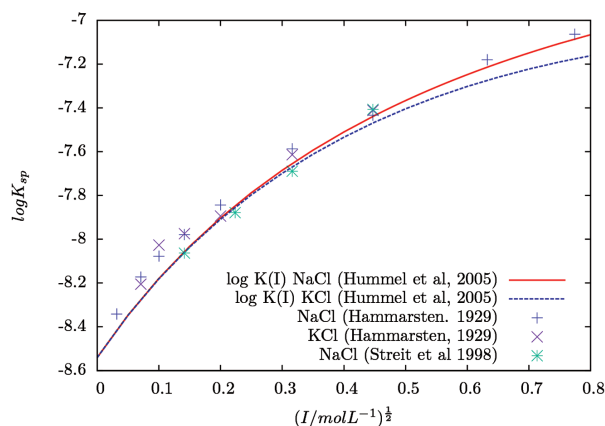
The solubility of CaOx in a 0.20 mol/L sodium chloride solution is  $1.94 \times 10^{-4}$  mol/L, while in an artificial urine solution this is increased to  $2.98 \times 10^{-4}$  mol/L (Streit et al. 1998).

For increasing concentrations of  $Mg^{2+}$  of 2.5, 5.0, and 7.5 mmol/L, the respective ion product values for calcium oxalate are  $1.0 \times 10^{-7}$ ,  $1.35 \times 10^{-7}$ , and  $2.02 \times 10^{-7}$  (Elliot and Ribeiro 1973). Thus, urine contains much higher concentrations of calcium and oxalate in solution than are present in a saturated solution of calcium oxalate in water. In addition to the dissolved salts, urine contains macromolecules. Several proteins and similar substances are secreted into the tubule by the tubular cells (Söhnel and Grases 1995; Tiselius 1997a; Højgaard and Tiselius 1999). Tamm-Horsfall Protein (THP) is the most abundant protein in human urine (Devuyst et al. 2005), with a concentration of the order of magnitude of  $10^{-7}$  mol/L in urine (Glauser et al. 2000; Laube et al. 2001); it therefore cannot bind a significant amount of calcium. While the macromolecules have sometimes been shown to promote crystal nucleation, they are also known to inhibit crystal growth (Rodgers et al. 1993). This process is mainly via the action of binding to calcium-rich centers on the crystal surface (Tiselius 2011b). Phytic acid, present at micromolar levels in urine, is another substance that has been shown to inhibit the growth of calcium oxalate crystals (Söhnel and Grases 1995), presumably by mechanisms akin to those mentioned above for macromolecules.

As already mentioned, the risk of stone formation can be determined from the supersaturated state of the stone forming salts. Methods involve measurement of ratios of concentrations of certain substances (Tiselius 1997b) and determining how much is required to initiate precipitation following the addition of the ions of interest to a sample of urine. For example, adding calcium chloride or ammonium oxalate induces CaOx precipitation (Luptak et al. 1994; Laube et al. 2000).

## QUANTITATIVE CHEMICAL SPECIATION MODELING

It is now widely accepted that the application of geochemical techniques to “predict, identify and quantify minerals in low temperature aqueous environments can be adapted” to the study of biofluids (Taunton et al. 2010). Thermodynamic calculations have been used routinely to investigate the state of saturation of substances in urine (Linder and Little 1986; Asplin et al. 1996; Parks et al. 1997; Laube et al. 2002; Königsberger and Tran-Ho 1997; Milosevic et al. 1998; Rodgers et al. 2006, 2011; Pak et al. 2009) providing a useful alternative to the induction of precipitation by substance addition. This technique uses measurements of substance concentrations to estimate free ion concentrations and



**FIGURE 6.** Ionic strength dependence of  $K_{sp}$  for COM at 37 °C. (Color online.)

supersaturation states so that risk can be evaluated.

The most widely used program to perform such calculations has been EQUIL. This program was developed by Finlayson in 1977 (Finlayson 1977; Brown and Purich 1992). EQUIL2 is an updated version of this program, which included translation from FORTRAN to BASIC, making it available on a larger number of computers (Werness et al. 1985). Enhancements led to a newer version, EQUIL93 (Brown et al. 1994), which increased the number of ions and complexes that could be represented and updated the thermodynamic database with data from the Martell and Smith critically evaluated compilation of equilibrium constants (Martell and Smith 1974–1982), and other sources.

A chemical speciation program (confusingly, also called EQUIL) was developed by Ting-Po and Nancollas in 1972 (Ting-Po and Nancollas 1972), but this program appears to be unrelated to that described above.

Another program frequently used in urolithiasis research is the Joint Expert Speciation System (JESS) software package (May and Murray 1991a, 1991b).

In addition to EQUIL and JESS, other software has also occasionally been used. Prywer and Mielniczek-Brzoska (2016) used HySS (Alderighi et al. 1999) to model chemical speciation in the formation of struvite kidney stones resulting from bacterial infection.

Grases et al. (1997) first used JESS to model the supersaturation of calcium and magnesium phosphates in artificial urine in 1997. In this work, citrate and oxalate were considered in addition to the inorganic salts. All possible complexes whose formation constants were available in the JESS thermodynamic database were thus considered. In addition, one of the then built-in activity coefficient models of JESS was used (Davies equation). Considering the number of species (213), reactions (265), and thermodynamic quantities (more than 4000, including enthalpy, free energy, and heat capacity values), this urine model was possibly the largest at that time. After incorporating solubility constants ( $\log K_{s0}$ ) determined in their laboratory (Streit et al. 1998), Königsberger and Tran-Ho (1997) employed this model to calculate solubilities of the three calcium oxalate hydrates in NaCl(aq) and urine-like liquors. Subsequently, the JESS urine model was extended to include uric acid and cystine (Königsberger and Königsberger 2001), resulting in a considerable increase in the number of species (280), reactions (380), and thermodynamic quantities (some 7200, mainly equilibrium constants but also standard potentials, Gibbs energies, enthalpies, and heat capacities). The effect of complexing species such as citrate and magnesium ions on calcium oxalate solubilities helped to identify conditions for reducing its supersaturation in urine (Königsberger and Tran-Ho 1997; Königsberger and Königsberger 2001). Significant effects of urine composition on uric acid (Königsberger and Wang 1999) and cystine (Königsberger et al. 2000) solubilities were not predicted nor found experimentally. Furthermore, the JESS modeling suggested regions of thermodynamic and kinetic control of calcium oxalate crystallization that correlated well with a clinical test (Grases et al. 2000).

To calculate the degree of saturation of a dissolved substance, values for the ion activity coefficients have to be determined. Several empirical models can be used for this. The Davies Equation 1 is an extension of Debye-Hückel theory without

adjustable parameters, it has no theoretical foundation, but often works fairly well for ionic strengths up to 0.1 mol/kg (Grenthe et al. 1997).

At 25 °C,

$$\log_{10} \gamma_i = -0.51 Z_i^2 \left( \frac{\sqrt{I_m}}{1 + \sqrt{I_m}} - 0.3 I_m \right) \quad (1)$$

where  $\gamma_i$  is the activity coefficient of ion  $i$ ;  $Z_i$  is the charge of ion  $i$ ;  $I_m$  is the ionic strength on molal scale.

Specific ion interaction theory (SIT) (Eq. 2) is a semi-empirical model based on Brønsted-Guggenheim-Scatchard models. It contains several parameters that have some theoretical basis (Grenthe et al. 1997).

$$\log_{10} \gamma_i = -\frac{Z_i^2 A \sqrt{I_m}}{1 + 1.5 \sqrt{I_m}} + \sum_k \varepsilon(i, k) m_k \quad (2)$$

where  $A$  is the Debye-Hückel parameter for activity coefficient;  $\varepsilon(i, k)$  are interaction coefficients for oppositely charged aqueous ions  $i$  and  $k$ ;  $m_k$  is the molality of ion  $k$ .

The current method used by JESS is the SIT-like equation, shown in Equation 3

$$\log_{10} K' = \log_{10} K^0 + \left( \frac{-\Delta Z^2 A \sqrt{I}}{1 + 1.5 \sqrt{I}} \right) + BI \quad (3)$$

where  $K^0$  is the equilibrium constant at infinite dilution;  $K'$  is the conditional equilibrium constant at finite ionic strength;  $A$  and  $\Delta Z^2$  are the Debye-Hückel parameter and a function of the ionic charges, respectively; and  $B$  is a temperature-dependent parameter (May 2000).

The JESS software package calculates  $\log(\text{SI})$  values:

$$\log(\text{SI}) = \log \frac{IAP}{K_{sp}}$$

where  $IAP$  is the ion-activity product,  $K_{sp}$  is the solubility product.

Using these methods, estimates of supersaturation of the calcium phosphate and calcium oxalate compounds have been calculated for final urine, as well as for the different nephron segments (Robertson and Nordin 1976; Luptak et al. 1994; Tiselius 1997; Rodgers et al. 2011; Robertson 2015). It has been determined that for the calcium phosphates, supersaturation and, therefore, the risk of crystallization is higher in the proximal and distal tubules (Luptak et al. 1994; Asplin et al. 1996; Tiselius 1997a; Rodgers et al. 2011; Robertson 2015). For calcium oxalate, supersaturation levels are higher in the collecting duct (Luptak et al. 1994; Rodgers et al. 2011; Robertson 2015). The variation in the values on which these calculations are based, as discussed above, indicates that the quantitative results from such calculations cannot be regarded as exact. In general, computational models should be used to gain insight into the working of a process, rather than in attempts to obtain individual numerical results that can be taken as the definitive answer to the problem (May 2015).

Using published data about concentrations of the solutes in the different nephron segments (Rodgers et al. 2011), shown in Table 3, some calculations performed using the JESS software package give the values shown in Table 4.

While some earlier work concentrated on the behavior of minerals under simulated lung fluid conditions, with a focus of assessing mineral durability and secondary mineral formation (Taunton et al. 2010), we prefer to emphasize instead the implications of Ostwald's Rule of Stages (Chung et al. 2009). This is known to work well for systems that reach equilibrium too rapidly to apply conventional reaction path analysis, in contrast to the long-term timeframe for minerals resident in the lungs.

The results in Table 4 indicate that brushite is the supersaturated substance with the lowest SI value under the conditions in the distal portion of the collecting duct and thus, brushite seems from Ostwald's Rule of Stages to be the substance most likely to precipitate. Brushite has indeed been found in some kidney stones (Grases and Costa-Bauza 2006), particularly in overgrowths of a calculus that had "plugged" the duct of Bellini (Evan et al. 2015). The core of that specimen contained hydroxyapatite, the most stable calcium phosphate phase, which may well have been formed by recrystallization of brushite. Another instance of stone plugging in the duct of Bellini contained COD (Grases et al. 2016), which is less stable than COM. Both of these stones were associated with renal tissue damage probably acting as heterogeneous nucleant. We conclude that the crystallization of metastable phases according to Ostwald's Rule of Stages can be applied to the growth of stones on "Randall's Plugs," which are usually associated with excessive supersaturation with respect to the stable phases (Khan and Canales 2015). The metastable phases brushite and COD were also found in cavities of low urodynamic efficacy, in which heterogeneous nucleants (organic matter and calcium phosphate crystals, respectively) become trapped and high supersaturation is maintained (Grases and Costa-Bauza 2006).

In contrast, the growth of papillary stones induced by Randall's Plaque inevitably proceeds even at the low supersaturation

prevailing in urine of normal composition. Such stones contain the stable phases HAP and COM (Grases et al. 2015; Grases et al. 2016). However, (metastable) amorphous calcium phosphates were found as possible precursors of Randall's Plaque (Evan 2010), which indicates high supersaturation and the applicability of Ostwald's Rule of Stages in interstitial tissue.

As a result of these findings, we have developed a model to calculate the concentration changes along the path of the nephron. The calculations are based on published values of concentration, reabsorption, and excretion of different substances within the sections of the nephron (Luptak et al. 1994; Asplin et al. 1996; Kok 1997; Tiselius 1997; Højgaard and Tiselius 1999; Rodgers et al. 2011, 2013). Output from the model for normal kidney filtration is shown in Table 5. The model allows different conditions to be investigated by changing input values that represent blood plasma concentrations of the substances under consideration and changes in how much of a particular substance is reabsorbed in a given nephron section. For example, it has been discovered that calcium oxalate stone formers often have reduced calcium reabsorption in the proximal tubule (Coe et al. 2011; Worcester et al. 2013), and the model allows simulation of such scenarios.

Using calculated concentrations for the different nephron sections log(SI) values for substances of interest can be determined using JESS. It should be stressed again that JESS calculates the chemical speciation, and hence log(SI), by considering all complex species whose formation constants are contained in its database.

Figure 7 shows log(SI) values for brushite for three different scenarios, normal kidney filtration with a plasma calcium concentration of 1.5 mmol/L and oxalate concentration of 1.75  $\mu$ mol/L, a high plasma calcium concentration of 3.0 mmol/L, and reduced calcium reabsorption in the proximal tubule together with the increased plasma calcium concentration. The second two situations result in an increased SI for the brushite all along the nephron. Log(SI) for brushite is above zero in the loop of Henle and the collecting duct, indicating an increased risk of precipitation in those locations.

Figure 8 shows the log(SI) values for the calcium oxalate monohydrate for the same three conditions described above. This shows an increased risk of precipitation toward the end of the nephron.

Figure 9 shows the log(SI) values for the calcium oxalate monohydrate for normal, a high plasma concentration of oxalate of 3.0  $\mu$ mol/L, and the same high value of plasma oxalate together with the reduction in reabsorption of calcium in the proximal tubule.

Both these simulations show log(SI) COM increasing in the proximal tubule to reach a peak in the ascending loop of Henle

**TABLE 3.** Nephron concentration data from Rodgers et al. (2011)

Substance	Concentration (mmol/L)						
	GF	PT	LH	DTp	DTd	CDm	CDd
Na <sup>+</sup>	135	135	278	79	93	94	109
K <sup>+</sup>	3.8	3.0	13.8	0.90	58.0	53.0	63.7
Ca <sup>2+</sup>	1.50	2.78	3.47	1.32	0.94	1.60	4.50
Mg <sup>2+</sup>	0.54	0.19	0.24	0.12	0.40	1.45	3.85
PO <sub>4</sub> <sup>3-</sup>	0.80	0.80	1.00	1.00	3.34	12.1	32.3
Oxalate	0.0015	0.01	0.013	0.013	0.04	0.12	0.32
Citrate	0.07	0.09	0.11	0.11	0.37	1.21	3.21
SO <sub>4</sub> <sup>2-</sup>	1.4	3.1	3.9	3.9	13.0	7.8	20.8
Cl <sup>-</sup>	139	139	293	101	145	146.6	170.0
pH	7.40	6.75	6.50–7.30	6.38–7.00	6.45–7.00	5.00–6.25	5.50–6.70
Avg. pH	7.40	6.75	6.90	6.69	6.725	5.625	6.1

**TABLE 4.** The log SI values for the stone forming salts

	pH	CaOx	Salt		
			Bru	HAP	OCF
GF	7.40	-1.267	-0.592	9.043	1.754
PT	6.75	-0.236	-0.458	7.365	1.118
LH	6.50	-0.291	-0.643	5.972	0.230
LH	7.30	-0.294	-0.516	9.546	2.208
DTp	6.38	-0.247	-0.726	4.413	-0.788
DTp	7.30	-0.256	-0.425	8.973	1.944
DTd	6.45	-0.145	-0.547	4.716	-0.328
DTd	7.00	-0.176	-0.400	7.294	1.183
CDm	5.00	0.506	-0.921	-1.694	-4.096
CDm	6.25	0.438	0.035	5.970	1.172
CDd	5.50	1.084	0.184	4.212	0.550
CDd	6.70	0.853	0.681	9.961	4.182

**TABLE 5.** Nephron concentration data from the model

Substance	Concentration (mmol/L)						
	GF	PT	LH	TAL	DT	CD	CX
Na <sup>+</sup>	145	146	324	121	129	22	124
K <sup>+</sup>	4.2	1.9	8.0	1.0	1.5	5.5	30.6
Ca <sup>2+</sup>	1.5	1.7	4.6	1.7	0.7	0.7	2.3
Mg <sup>2+</sup>	0.4	0.7	2.3	0.6	0.9	0.3	1.7
PO <sub>4</sub> <sup>3-</sup>	1.5	1.0	1.5	1.5	2.2	3.6	20
Oxalate	0.002	0.003	0.001	0.01	0.02	0.03	0.2
Citrate	0.3	0.09	0.11	0.11	0.37	1.21	3.21
SO <sub>4</sub> <sup>2-</sup>	0.35	0.42	0.8	0.8	1.2	1.9	10.5
Cl <sup>-</sup>	125	153	197	144	142	20	112
pH	7.4	6.75	7.0	7.0	6.45	6.25	6.0

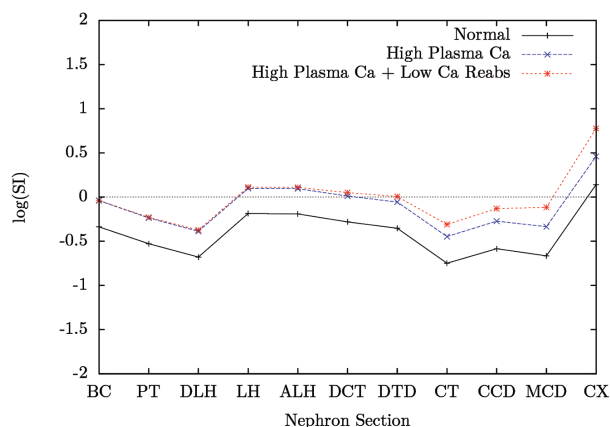


FIGURE 7. Log(SI) brushite with increased calcium. (Color online.)

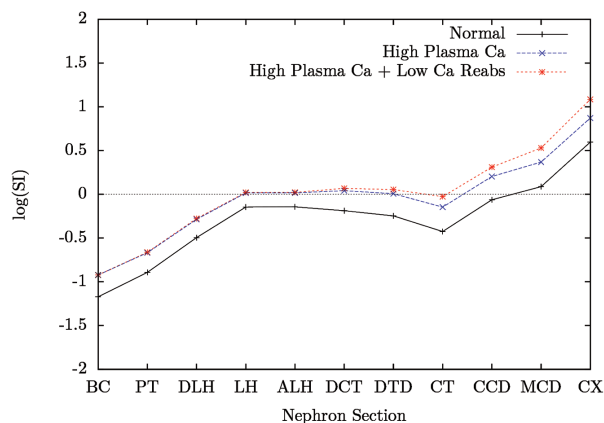


FIGURE 8. Log(SI) COM with increased calcium. (Color online.)

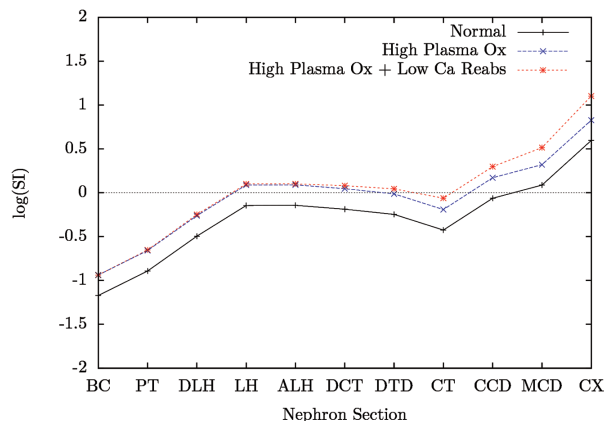


FIGURE 9. Log(SI) COM with increased oxalate. (Color online.)

before decreasing toward the distal tubule, and then increasing steadily in the collecting duct. The risk of crystal formation, where  $\log(\text{SI}) > 0$ , is only seen with higher than normal calcium or oxalate plasma levels, and increased with a pathological reabsorption profile. This is in good agreement with the results of Robertson (2015).

JESS Version 8.3 used in this work leads to the same general conclusion as the previous work by Rodgers et al. (Rodgers et al. 2011), although there are small quantitative differences due to changes in the way weak ion associations are handled (May 2015). The absolute values of the saturations calculated by different JESS versions change to a small extent over time, but in almost all cases their pattern through the different compartments is the same and no large discrepancies have been found. It can thus be concluded that the changes are due to updates that have been made to the database. Further information about how JESS approaches the selection of equilibrium constants is given in The JESS Primer, available via the web site <http://jess.murdoch.edu.au> (May 2015).

However, this issue is complicated by several theoretical and practical factors. These include the fact that protein binding of calcium ions is still not well characterized (Taunton et al. 2010; Holt et al. 2014), calcium buffering and the observation that coating of seeds by proteins can cause inhibition of crystal growth. Another factor is that the saturation state of relevant minerals may alter under physiological conditions (Miller et al. 1958; Streit et al. 1998). Note also that in this regard chemical speciation calculations using ion association frameworks have well known limitations (May 2015). The *absolute* SI values calculated by JESS are therefore interesting but need to be interpreted with caution. However, it is clear that their *changes* across the nephron are significant and must be taken into account.

## IMPLICATIONS

Kidney stone formation is a serious medical problem for which the underlying mechanisms are poorly understood. In the human body, hydroxyapatite must be kept supersaturated to allow the processes that form bones and teeth to operate. As a result of this, several calcium phosphate minerals tend to be supersaturated in various biofluids. A delicate biological balance is therefore required between preventing the formation of solid structures where and when they are harmful and producing them as required.

Understanding kidney stone formation requires the investigation of mineral formation in a complex environment, where the changes that are taking place are often very difficult to observe directly. Interdisciplinary research in particular can be of great value in medical science. Combining techniques from biology, geochemistry, thermodynamics, mathematics, and computer science, comprehensive models can now be developed to investigate and explain processes taking place in the human body. Computer modeling techniques are a powerful tool that can be utilized to overcome the problems with experimental in vivo and in vitro investigations. Thermodynamic calculations have been shown to be useful, especially in improving understanding of the processes involved in kidney stone formation. Much insight can be gained into the processes taking place and the interactions between them. As more and better data are included in the databases that these computer models use, the results obtained from the models can be expected to improve. Basic mineralogical theory and experiments provide the pre-requisite building blocks for these databases. The modeling is then able to combine theory and experiment to simulate the complex interactions between the components of the system being investigated. Similar issues arise



in geochemical complex aqueous environments, where meta-stable equilibria and kinetic restrictions often prevail. Insights obtained by geochemical modelers may therefore also be helpful in improving the computational area of kidney stone research.

Determining the details of the initial solid phase formation in the loop of Henle, or distal tubule, would probably be of greatest value as this information would help to show how the risk of this initial particle formation can be reduced. Thus, investigation of crystal seed formation is likely to be a key area for future research. Better understanding of the thermodynamics, kinetics, and morphology of the minerals involved is therefore needed to improve prospects in this medical arena.

## ACKNOWLEDGMENTS

The authors thank the reviewers Allen Rodgers and Greg Druschel for their suggestions for the improvement of this manuscript.

## REFERENCES CITED

- Abdel-Halim, R. (2005) Urolithiasis in adults: Clinical and biochemical aspects. *Saudi Medical Journal*, 26, 705–713.
- Alderighi, L., Gans, P., Ienco, A., Peters, D., Sabatini, A., and Vacca, A. (1999) Hyperquad simulation and speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially soluble species. *Coordination Chemistry Reviews*, 184, 311–318.
- Asplin, J.R., Mandel, N.S., and Coe, F.L. (1996) Evidence for calcium phosphate supersaturation in the loop of Henle. *American Journal of Physiology*, 270, F604–F613.
- Atherton, J.C. (2006a) Function of the nephron and the formation of urine. *Anaesthesia and Intensive Care Medicine*, 7, 221–226.
- (2006b) Renal blood flow, glomerular filtration and plasma clearance. *Anaesthesia and Intensive Care Medicine*, 7, 216–220.
- Bell, G.H., Davidson, J.N., and Scarborough, H. (1968) *Textbook of Physiology and Biochemistry*, 7th ed. Livingstone, London.
- Brown, C.M., and Purich, D.L. (1992) Physical-chemical processes in kidney stone formation. In F.L. Coe and M.J. Favus, Eds., *Disorders of Bone and Mineral Metabolism*, chapter 29, p. 613–624. Raven Press, New York.
- Brown, C.M., Ackerman, D.K., and Purich, D.L. (1994) EQUIL93: A tool for experimental and clinical urolithiasis. *Urological Research*, 22, 119–126.
- Chung, S.-Y., Kim, Y.-M., Kim, J.-G., and Kim, Y.-J. (2009) Multiphase transformation and Ostwald's rule of stages during crystallization of a metal phosphate. *Nature Physics*, 5, 68–73.
- Coe, F.L., Evan, A., and Worcester, E. (2011) Pathophysiology-based treatment of idiopathic calcium kidney stones. *Clinical Journal of the American Society of Nephrology*, 6, 2083–2092.
- Daniele, P.G., Sonogo, S., Rozani, M., and Marangella, M. (1985) Ionic strength dependence of formation constants. Part 8. Solubility of calcium oxalate monohydrate and calcium hydrogenphosphate dihydrate in aqueous solution, at 37°C and different ionic strengths. *Annali di Chimica*, 75, 245–251.
- Devuyst, O., Dahan, K., and Pirson, Y. (2005) Tamm-Horsfall protein or uromodulin: new ideas about an old molecule. *Nephrology Dialysis Transplantation*, 20, 1290–1294.
- Diem, K., and Lentner, C., Eds. (1970) *Documenta Geigy Scientific Tables*, 7th ed. J.R. Geigy SA, Basle, Switzerland.
- Eisner, B.H., Eisenberg, M.L., and Stoller, M.L. (2010) The relationship between body mass index and quantitative 24-hour urine chemistries in patients with nephrolithiasis. *Urology*, 75, 1289–1293.
- Elliot, J.S., and Ribeiro, M.E. (1973) The effect of varying concentrations of calcium and magnesium upon calcium oxalate solubility. *Investigative Urology*, 10, 295–297.
- Evan, A.P. (2010) Physiopathology and etiology of stone formation in the kidney and the urinary tract. *Pediatric Nephrology*, 25, 831–841.
- Evan, A.P., Worcester, E.M., Coe, F.L., Williams, J.J., and Lingeman, J.E. (2015) Mechanisms of human kidney stone formation. *Urolithiasis*, 43 (Supplement 1), S19–S32.
- Finlayson, B. (1977) Calcium stones: Some physical and clinical aspects. In D.S. David, Ed., *Calcium Metabolism in Renal Failure and Nephrolithiasis Perspectives in nephrology and hypertension*, chapter 10, p. 337–382. Wiley, New York.
- (1978) Physicochemical aspects of urolithiasis. *Kidney International*, 13, 344–360.
- Finlayson, B., and Reid, F. (1978) The expectation of free and fixed particles in urinary stone disease. *Investigative Urology*, 15, 442–448.
- Finlayson, B., Khan, S.R., and Hackett, R.L. (1990) Theoretical chemical models of urinary stones. In J.E.A. Wickham and A.C. Buck, Eds., *Renal Tract Stone*, chapter 10, p. 133–147. Churchill Livingstone, Edinburgh.
- Gamsjäger, H., and Königsberger, E. (2003) Solubility of sparingly soluble ionic solids in liquids. In G. Hefter and R. Tomkins, Eds., *The Experimental Determination of Solubilities*, chapter 4.2, p. 315–358. Wiley, Chichester, U.K.
- Glauser, A., Hochreiter, W., Jaeger, P., and Hess, B. (2000) Determinants of urinary excretion of Tamm-Horsfall protein in non-selected kidney stone formers and healthy subjects. *Nephrology Dialysis Transplantation*, 15, 1580–1587.
- Grases, F., and Costa-Bauza, A. (2006) Mechanisms of renal and salivary calculi formation and development. In E. Königsberger and L.-C. Königsberger, Eds., *Biomimetalization—Medical Aspects of Solubility*, chapter 2, p. 39–69. Wiley, Chichester, U.K.
- Grases, F., Villacampa, A.I., Söhnel, O., Königsberger, E., and May, P.M. (1997) Phosphate composition of precipitates from urine-like liquors. *Crystal Research and Technology*, 32, 707–715.
- Grases, F., Costa-Bauza, A., and Garcia-Ferragut, L. (1998) Biopathological crystallization: a general view about the mechanisms of renal stone formation. *Advances in Colloid and Interface Science*, 74, 169–194.
- Grases, F., Söhnel, O., and Costa-Bauza, A. (1999) Renal stone formation and development. *International Urology and Nephrology*, 31, 591–600.
- Grases, F., Costa-Bauza, A., Königsberger, E., and Königsberger, L.-C. (2000) Kinetic versus thermodynamic actors in calcium renal lithiasis. *International Urology and Nephrology*, 32, 19–27.
- Grases, F., Costa-Bauza, A., Gomila, I., Ramis, M., Garcia-Raja, A., and Prieto, R.M. (2012) Urinary pH and renal lithiasis. *Urological Research*, 40, 41–46.
- Grases, F., Costa-Bauza, A., Bonarriba, C.R., Pieras, E.C., Fernández, R.A., and Rodríguez, A. (2015) On the origin of calcium oxalate monohydrate papillary renal stones. *Urolithiasis*, 43 (Supplement 1), S33–S39.
- Grases, F., Söhnel, O., and Costa-Bauza, A., Servera, A., and Benezam, J. (2016) A case of Randall's Plugs associated to calcium oxalate dihydrate calculi. *Urology Case Reports*, 7, 37–38.
- Grenthe, I., Plyasunov, A.V., and Spahiu, K. (1997) Modelling in aquatic chemistry. In I. Grenthe and I. Puigdomenech, editors, *Modelling in Aquatic Chemistry*, chapter IX, p. 325–426. Nuclear Energy Agency, Organisation for Economic Co-operation and Development.
- Gutzow, I., Atanassova, S., Budevsky, G., and Neykov, K. (1993) Solubility, inhibited growth and dissolution kinetics of calcium oxalate crystals in solutions, containing hippuric acid. *Urological Research*, 21, 181–185.
- Guyton, A.C., and Hall, J.E. (2000) *Textbook of Medical Physiology*, 10th ed. WB Saunders Company, Philadelphia.
- Hammarsten, G. (1929) On calcium oxalate and its solubility in the presence of inorganic salts with special reference to the occurrence of oxaluria. *Comptes Rendus des Travaux du Laboratoire Carlsberg*, 17, 1–85.
- Hesse, A., Classen, A., Knoll, M., Timmermann, F., and Vahlensieck, W. (1986) Dependence of urine composition on the age and sex of healthy subjects. *Clinica Chimica Acta*, 160, 79–86.
- Hodgkinson, A. (1980) Solubility of calcium oxalate in human urine, simulated urine, and water. *Investigative Urology*, 18, 123–126.
- Højgaard, I., and Tiselius, H.-G. (1999) Crystallization in the nephron. *Urological Research*, 27, 397–403.
- Holt, C., Lenton, S., Nylander, T., Sørensen, E.S., and Teixeira, S.C. (2014) Mineralisation of soft and hard tissues and the stability of biofluids. *Journal of Structural Biology*, 185, 383–396.
- Hughes, P. (2007) Kidney stones epidemiology. *Nephrology*, 12, S26–S30.
- Hummel, W., Anderegg, G., Rao, L., Puigdomenech, I., and Tochiyama, O. (2005) *Chemical Thermodynamics of Compounds and Complexes of U, Np, Pu, Am, Tc, Se, Ni and Zr with Selected Organic Ligands*. OECD-NEA, Elsevier, Amsterdam.
- Kerr, J. (1999) *Atlas of Functional Histology*. Mosby, London.
- Khan, S.R., and Canales, B.K. (2015) Unified theory on the pathogenesis of Randall's plaques and plugs. *Urolithiasis*, 43 (Supplement 1), S109–S123.
- Knight, J., Jaing, J., Assimos, D., and Holmes, R. (2006) Hydroxyproline ingestion and urinary oxalate and glycolate excretion. *Kidney International*, 70, 1929–1934.
- Kok, D.J. (1997) Intratubular crystallization events. *World Journal of Urology*, 15, 219–228.
- Kok, D.J., and Khan, S.R. (1994) Calcium oxalate nephrolithiasis, a free or fixed particle disease. *Kidney International*, 46, 847–854.
- Königsberger, E., and Königsberger, L.-C. (2001) Thermodynamic modeling of crystal deposition in humans. *Pure and Applied Chemistry*, 73, 785–797.
- (2006) Solubility phenomena related to normal and pathological biomimetalization processes. In E. Königsberger and L.-C. Königsberger, Eds., *Biomimetalization—Medical Aspects of Solubility*, chapter 1, p. 1–37. Wiley, Chichester, U.K.
- Königsberger, E., and Tran-Ho, L.-C. (1997) Solubility of substances related to urolithiasis—experiments and computer modelling. *Current Topics in Solution Chemistry*, 2, 183–202.
- Königsberger, E., and Wang, Z. (1999) Solubility of uric acid in salt solutions and artificial urine. *Monatshefte für Chemie*, 130, 1067–1073.
- Königsberger, E., Wang, Z., and Königsberger, L.-C. (2000) Solubility of L-Cystine in NaCl and artificial urine solutions. *Monatshefte für Chemie*, 131, 39–45.

- Kumar, S., and Muchmore, A. (1990) Tamm-Horsfall protein-uromodulin (1950-1990) *Kidney International*, 37, 1395-1401.
- Laube, N., Schneider, A., and Hesse, A. (2000) A new approach to calculate the risk of calcium oxalate crystallization from unprepared native urine. *Urological Research*, 28, 274-280.
- Laube, N., Glatz, S., and Hesse, A. (2001) The relation of urinary Tamm-Horsfall-Protein on CaOx-crystallization under the scope of the Bonn-Risk-Index. *Urological Research*, 29, 45-49.
- Laube, N., Labedzke, V., Hergarten, S., and Hesse, A. (2002) Determination of urinary calcium-oxalate formation risk with BONN-Risk-Index and EQUIL applied to a family. *Journal of Chemical Information and Computer Sciences*, 42, 633-639.
- Linder, P.W., and Little, J.C. (1986) Prediction by computer modelling of the precipitation of stone-forming solids from urine. *Inorganica Chimica Acta*, 123, 137-145.
- Luptak, J., Bek-Jensen, H., Fornander, A.-M., Hojgaard, I., Nilsson, M.-A., and Tiselius, H. (1994) Crystallization of calcium oxalate and calcium phosphate at supersaturation levels corresponding to those in different parts of the nephron. *Scanning Microscopy*, 8, 47-62.
- Martell, A.E., and Smith, R.M. (1974-1982) Critical Stability Constants, vols. 1-6. Plenum Press, New York.
- May, P.M. (2000) A simple, general and robust function for equilibria in aqueous electrolyte solutions to high ionic strength and temperature. *Chemical Communications*, 1265-1266.
- (2015) JESS at thirty: Strengths, weaknesses and future needs in the modelling of chemical speciation. *Applied Geochemistry*, 55, 3-16.
- May, P., and Murray, K. (1991a) JESS, A Joint Expert Speciation System—I. Raison d'être. *Talanta*, 38, 1409-1417.
- (1991b) JESS, A Joint Expert Speciation System—II. The thermodynamic database. *Talanta*, 38, 1419-1426.
- Miller, G.H., Vermeulen, C.W., and Moore, J.D. (1958) Calcium oxalate solubility in urine: Experimental urolithiasis XIV. *The Journal of Urology*, 79, 607-612.
- Milosevic, D., Batinic, D., Konjevoda, N.B.P., Stambuk, N., Votava-Raic, A., Fumic, V.B.K., Rumenjak, V., Stavljenic-Rukavina, A., Nizic, L., and Vrljickak, K. (1998) Determination of urine supersaturation with computer program Equil 2 as a method for estimation of the risk of urolithiasis. *Journal of Chemical Information and Computer Sciences*, 38, 646-650.
- Moe, O.W. (2006) Kidney stones: pathophysiology and medical management. *The Lancet*, 367, 333-344.
- Moran, M.E. (2014) Urolithiasis: A Comprehensive History. Springer, New York.
- Pak, C.Y.C., Maalouf, N.M., Rodgers, K., and Poindexter, J.R. (2009) Comparison of semi-empirical and computer derived methods for estimating urinary saturation of calcium oxalate. *The Journal of Urology*, 182, 2951-2956.
- Parks, J.H., and Coe, F.L. (1996) The financial effects of kidney stone prevention. *Kidney International*, 50, 1706-1712.
- Parks, J.H., Coward, M., and Coe, F.L. (1997) Correspondence between stone composition and urine supersaturation in nephrolithiasis. *Kidney International*, 51, 894-900.
- Prywer, J., and Mielniczek-Brzoska, E. (2016) Chemical equilibria of complexes in urine. a contribution to the physicochemistry of infectious urinary stone formation. *Fluid Phase Equilibria*, 425, 282-288.
- Rendina, D., De Filippo, G., De Pascale, F., Zampa, G., Muscarello, R., De Palma, D., Ippolito, R., and Strazzullo, P. (2013) The changing profile of patients with calcium nephrolithiasis and the ascendancy of overweight and obesity: a comparison of two patient series observed 25 years apart. *Nephrology Dialysis Transplantation*, 28, iv146-iv151.
- Robertson, W.G. (2004) Kidney models for calcium oxalate stone formation. *Nephron Physiology*, 98, 21-30.
- (2015) Potential role of fluctuations in the composition of renal tubular fluid through the nephron in the initiation of Randall's plugs and calcium oxalate crystalluria in a computer model of renal function. *Urolithiasis*, 43(Supplement 1), S93-S107.
- Robertson, W., and Nordin, B. (1976) Physio-chemical factors governing stone formation. In D.I. Williams and G.D. Chisholm, Eds., *Scientific Foundations of Urology*, chapter 37, p. 254-267. Heinemann Medical, London.
- Robertson, W.G., Scurr, D.S., and Bridge, C.M. (1981) Factors influencing the crystallisation of calcium oxalate in urine—critique. *Journal of Crystal Growth*, 53, 182-194.
- Rodgers, A. (2006) The riddle of kidney stone disease: lessons from Africa. *Urological Research*, 34, 92-95.
- Rodgers, A.L., Ball, D., and Harper, W. (1993) Urinary macromolecules are promoters of calcium oxalate nucleation in human urine: turbidimetric studies. *Clinica Chimica Acta*, 220, 125-134.
- Rodgers, A., Allie-Hamdulay, S., and Jackson, G. (2006) Therapeutic action of citrate in urolithiasis explained by chemical speciation: increase in pH is the determinant factor. *Nephrology Dialysis Transplantation*, 21, 361-369.
- Rodgers, A.L., Allie-Hamdulay, S., Jackson, G., and Tiselius, H.-G. (2011) Simulating calcium salt precipitation in the nephron using chemical speciation. *Urological Research*, 39, 245-251.
- Rodgers, A.L., Allie-Hamdulay, S., Jackson, G.E., and Durbach, I. (2013) Theoretical modeling of the urinary supersaturation of calcium salts in healthy individuals and kidney stone patients: Precursors, speciation and therapeutic protocols for decreasing its value. *Journal of Crystal Growth*, 382, 67-74.
- Romero, V., Akpinar, H., and Assimos, D.G. (2010) Kidney stones: A global picture of prevalence, incidence, and associated risk factors. *Reviews in Urology*, 12, e86-e96.
- Saude, E.J., Adamko, D., Rove, B.H., Marrie, T., and Sykes, B.D. (2007) Variation of metabolites in normal human urine. *Metabolomics*, 3, 439-451.
- Sawada, K. (1997) The mechanisms of crystallization and transformation of calcium carbonates. *Pure and Applied Chemistry*, 69, 921-928.
- Siener, R., Jähnen, A., and Hesse, A. (2004) Influence of a mineral water rich in calcium, magnesium and bicarbonate on urine composition and the risk of calcium oxalate crystallization. *European Journal of Clinical Nutrition*, 58, 270-276.
- Söhnel, O., and Grases, F. (1995) Calcium oxalate monohydrate renal calculi. formation and development mechanism. *Advances in Colloid and Interface Science*, 59, 1-17.
- (2011) Supersaturation of body fluids, plasma and urine, with respect to biological hydroxyapatite. *Urological Research*, 39, 429-436.
- Soucie, J.M., Thun, M.J., Coates, R.J., William, M., and Austin, H. (1994) Demographic and geographic variability of kidney stones in the United States. *Kidney International*, 46, 893-899.
- Streit, J., Tran-Ho, L.-C., and Königsberger, E. (1998) Solubility of the three calcium oxalate hydrates in sodium chloride solutions and urine-like liquors. *Monatshefte für Chemie*, 129, 1225-1236.
- Taunton, A.E., Gunter, M.E., Druschel, G.K., and Wood, S.A. (2010) Geochemistry in the lung: Reaction-path modeling and experimental examination of rock-forming minerals under physiologic conditions. *American Mineralogist*, 95, 1624-1635.
- Taylor, E.N., and Curhan, G.C. (2007) Differences in 24-hour urine composition between black and white women. *Journal of the American Society of Nephrology*, 18, 654-659.
- Taylor, E.N., Stampfer, M.J., Mount, D.B., and Curhan, G.C. (2010) DASH-Style diet and 24-hour urine composition. *Clinical Journal of the American Society of Nephrology*, 5, 2315-2322.
- Thomas, B., and Hall, J. (2005) Urolithiasis. *Surgery*, 23(4), 129-133.
- Ting-Po, I., and Nancollas, G.H. (1972) EQUIL—a general computational method for the calculation of solution equilibria. *Analytical Chemistry*, 44, 1940-1950.
- Tiselius, H.G. (1997a) Estimated levels of supersaturation with calcium phosphate and calcium oxalate in the distal tubule. *Urological Research*, 25, 153-159.
- (1997b) Risk formulas in calcium oxalate urolithiasis. *World Journal of Urology*, 15, 175-186.
- (2011a) A hypothesis of calcium stone formation: an interpretation of stone research during the past decades. *Urological Research*, 39, 231-243.
- (2011b) Who forms stones and why? *European Urology Supplements*, 10, 408-414.
- (2015) Should we modify the principles of risk evaluation and recurrence preventive treatment of patients with calcium oxalate stone disease in view of the etiologic importance of calcium phosphate? *Urolithiasis*, 43, S47-S57.
- Tiselius, H., Lindbäck, B., Fornander, A.-M., and Nilsson, M.-A. (2009) Studies on the role of calcium phosphate in the process of calcium oxalate crystal formation. *Urological Research*, 37, 181-192.
- Tomazic, B.B., and Nancollas, G.H. (1980) The kinetics of dissolution of calcium oxalate hydrates. *Investigative Urology*, 18, 97-101.
- Werness, P.G., Duckworth, S.C., and Smith, L.H. (1979) Calcium oxalate hydrate crystal growth. *Investigative Urology*, 17, 230-233.
- Werness, P.G., Brown, C.M., Smith, L.H., and Finlayson, B. (1985) EQUIL2: A BASIC computer program for the calculation of urinary saturation. *The Journal of Urology*, 134, 1242-1244.
- Williams, H.E. (1978) Oxalic acid and the hyperoxaluric syndromes. *Kidney International*, 13, 410-417.
- Worcester, E.M., Bergsland, K.J., Gillen, D.L., and Coe, F.L. (2013) Evidence for increased renal tubule and parathyroid gland sensitivity to serum calcium in idiopathic hypercalcaemia. *American Journal of Physiology. Renal Physiology*, 305(6), F853-F860.

MANUSCRIPT RECEIVED MARCH 23, 2016

MANUSCRIPT ACCEPTED NOVEMBER 21, 2016

MANUSCRIPT HANDLED BY MICKEY GUNTER